

Sub 2
genome of the mammalian host cell, so that the regulatory element is operatively associated with an endogenous gene of the mammalian host cell and so that activation and expression of the endogenous gene are controlled by the exogenous regulatory element.--

QA 1 Cont
✓ --27. (New) The mammalian host cell of Claim 26 further having an exogenous amplifiable gene operatively associated with the modified gene of Claim 1 so that the modified gene is also amplifiable.--

✓ --28. (New) The mammalian host cell of Claim 26 or 27 wherein the modified gene contains at least one mutation introduced via the homologous recombination of the exogenous nucleotide element into the genome of the mammalian host cell.--

✓ --29. (New) The mammalian host cell of Claim 28 wherein the mutation occurs within the amino acid coding region of the endogenous gene.--

✓ --30. (New) The mammalian host cell of Claim 28 wherein the mutation occurs within the 3' untranslated region of the endogenous gene.--

✓ --31. (New) The mammalian host cell of Claim 28 wherein the mutation occurs within the 5' region of the endogenous gene.--

AmB3
✓ --32. (New) The mammalian host cell of Claim 27 wherein the exogenous amplifiable gene is integrated, via homologous recombination, into the mammalian host cell genome.--

B
✓ --33. (New) The mammalian host cell of Claim 32, *or 72* wherein the endogenous gene contains at least one mutation introduced via the homologous recombination of the exogenous amplifiable gene.--

AmB3
✓ --34. (New) The mammalian host cell of Claim 33 wherein the mutation occurs within the amino acid coding region of the endogenous gene.--

✓ --35. (New) The mammalian host cell of Claim 33 wherein the mutation occurs within the 3' untranslated region of the endogenous gene.--

✓ --36. (New) The mammalian host cell of Claim 33 wherein the mutation occurs within the 5' region of the endogenous gene.--

Page 34
✓--37. (New) The mammalian host cell of Claim 26 or 27 wherein the mammalian cell does not grow readily in culture.--

✓--38. (New) The mammalian host cell of Claim 26 or 27 wherein the mammalian host cell is a primate cell.--

✓-39. (New) The primate cell of Claim 38 wherein the primate cell is a human cell.--

*pp
ant*
✓-40. (New) The human cell of Claim 39 wherein the human cell is a normal cell or a neoplastic cell.--

✓-41. (New) The human cell of Claim 39 wherein the human cell is a somatic or a germ cell.--

✓--42. (New) The human cell of Claim 39 wherein the human somatic cell is a diploid skin fibroblast cell.--

3
✓--43. (New) The mammalian host cell of Claim 27^{or 32} wherein the amplifiable exogenous gene is dihydrofolate reductase, metallothionein-I, metallothionein-II, adenosine deaminase, glutamine synthetase, or ornithine decarboxylase.--

✓ --44. (New) A secondary expression host cell
having the modified gene of Claim 26.--

✓ --45. (New) A secondary expression host cell
having the modified gene and the amplifiable gene of Claim
27.--

✓ --46. (New) The secondary expression host cell
of Claim 44 ~~or 45~~ wherein the secondary expression host cell
is a mammalian cell.--

✓ --47. (New) The mammalian cell of Claim 46
wherein the mammalian cell is a Chinese hamster ovary cell,
a monkey kidney cell, a C127 mouse fibroblast cell, a 3T3
mouse cell, a Vero cell, or a 293 cell.--

✓ --48. (New) A method for producing a mammalian
host cell having a modified gene comprising:

(a) transforming a mammalian host cell with a
nucleotide sequence comprising an exogenous nucleotide
regulatory element flanked by a nucleotide sequence
homologous to a region of at least about 150 nucleotides of
an endogenous gene present in the mammalian host cell so
that the nucleotide regulatory element is integrated via

Am 85
homologous recombination into the genome of the mammalian host cell; and

(b) selecting a transformed mammalian host cell having the modified gene in which the exogenous nucleotide regulatory element is operatively associated with the endogenous gene so that activation and expression of the endogenous gene are controlled by the exogenous regulatory element.--

Ag
ent
✓ --49. (New) A method for producing a mammalian host cell having an amplifiable modified gene comprising:

(c) transforming a mammalian host cell with a nucleotide sequence comprising an exogenous nucleotide regulatory element, an exogenous amplifiable gene, and a nucleotide sequence homologous to a region of at least about 150 nucleotides of an endogenous gene present in the mammalian host cell so that the exogenous nucleotide regulatory element and the exogenous amplifiable gene are integrated via homologous recombination into the genome of the mammalian host cell; and

(d) selecting a transformed mammalian host cell having the amplifiable modified gene in which the exogenous nucleotide regulatory element and the exogenous amplifiable

gene are operatively associated with the endogenous gene so that activation and expression of the endogenous gene are controlled by the exogenous regulatory element, and the amplifiable gene is operatively associated with the endogenous gene and the exogenous nucleotide regulatory element so that the endogenous gene and the exogenous nucleotide regulatory region are also amplifiable.--

CB ✓ --50. (New) The method of Claim 48, ^{on 49} ~~or 49~~ *75 49 0076*
wherein the nucleotide sequence used to transform the mammalian host cell further comprises a selectable marker and selecting a transformed mammalian host cell further comprises selecting a transformed mammalian host cell having the selectable marker.--

A1a
Ant
✓ --51. (New) The method of Claim 48 or 49 wherein the mammalian host cell does not grow readily in culture.--

B ✓ --52. (New) The method of Claim 48, ^{on 49} ~~or 49~~ *75 49 0076*
wherein the mammalian host cell is a primate cell.--

✓ --53. (New) The method of Claim 52 wherein the primate cell is a human cell.--

✓-54. (New) The method of Claim 53 wherein the human cell is a normal cell or a neoplastic cell.--

✓--55. (New) The method of Claim 53 wherein the human cell is a somatic or a germ cell.--

✓--56. (New) The method of Claim 55 wherein the human somatic cell is a diploid skin fibroblast cell.--

Q1 C B
cm
✓--57. (New) The method of Claim ^{75, 49 or 76} 49 wherein the amplifiable exogenous gene is dihydrofolate reductase, metallothionein-I, metallothionein-II, adenosine deaminase, glutamine synthetase, or ornithine decarboxylase.--

Sub C B
✓--58. (New) The method of Claim ^{75, 49 or 76} 48 _{or 49} wherein the exogenous flanking homologous nucleotide element introduces at least one mutation into the endogenous gene upon integration via homologous recombination into the genome of the mammalian host cell.--

✓--59. (New) The method of Claim 58 wherein the mutation in the endogenous gene occurs within the amino acid coding region of the gene.--

✓--60. (New) The method of Claim 58 wherein the mutation in the endogenous gene occurs within the 3' untranslated region of the gene.--

✓--61. (New) The method of Claim 58 wherein the mutation in the endogenous gene occurs in the 5' region of the gene.--

✓--62. (New) A method for producing a secondary expression host cell which expresses the modified gene of Claim 26, comprising:

(e) transforming a secondary expression host cell with nucleic acid isolated from the mammalian host cell of Claim 26; and

(f) selecting a transformed secondary expression host cell which expresses the modified gene of Claim 1.--

✓--63. (New) A method for producing a secondary host cell having the amplifiable modified gene of Claim 27 comprising:

(g) transforming a secondary expression host cell with nucleic acid isolated from the mammalian host cell of Claim 27; and

Auth 67
B
(h) selecting a transformed secondary expression host cell which contains the amplifiable gene of Claim 27 and expresses the amplifiable modified gene of Claim 27.--

QA
cont
B
--64. (New) The method of Claim 62, ^{82, 63, 64, 65} ~~62, 63~~ wherein the nucleotide sequence used to transform the secondary expression host cell further comprises a selectable marker and selecting a transformed secondary expression host cell further comprises selecting a transformed secondary expression host cell having the selectable marker.--

B
✓ --65. (New) The method of Claim 62, ^{82, 63, 64, 65} ~~62, 63~~ wherein the secondary host cell is a mammalian cell.--

✓ --66. (New) The method of Claim 65 wherein the mammalian cell is a Chinese hamster ovary cell, a monkey kidney cell, a C127 mouse fibroblast cell, a 3T3 mouse cell, a Vero cell, or a 293 cell.--

✓ --67. (New) A method for activating an endogenous gene in a mammalian cell comprising:

(i) transforming a mammalian cell with a nucleotide sequence comprising an exogenous nucleotide regulatory element flanked by a nucleotide sequence homologous to a region of at least about 150 nucleotides of an endogenous gene present in the mammalian cell so that the nucleotide regulatory element is integrated via homologous recombination into the genome of the mammalian cell; and

(j) selecting a transformed mammalian cell in which the exogenous nucleotide regulatory element is operatively associated with the endogenous gene so that the endogenous gene is activated by the exogenous regulatory element and the endogenous gene expresses its gene product.--

✓ --68. (New) The method of Claim 67 wherein the mammalian cell is a Chinese hamster ovary cell, a monkey kidney cell, a C127 mouse cell, a 3T3 mouse cell, a Vero cell, or a 293 cell.--

✓ --69. (New) A method for producing a recombinant protein comprising culturing a mammalian host cell having a modified gene comprising an exogenous nucleotide regulatory element operatively associated, via homologous recombination into the genome of the mammalian host cell, with an endogenous gene of the mammalian host cell so that activation and expression of the endogenous gene are controlled by the exogenous regulatory element, under conditions wherein the modified gene is activated and the recombinant protein encoded by the modified gene is produced.--

✓ --70. (New) The method of Claim 69 further comprising isolating the recombinant protein produced by culturing the mammalian host cell.--

✓ --71. (New) A recombinant protein produced by the method of Claim 69.--

PATENT
DOCKET NO. 3.2

Respectfully submitted,

By SaraLynn Mandel
SaraLynn Mandel
Reg. No. 31,853

Dated: 8/5/93

CELL GENESYS, INC.
322 Lakeside Drive
Foster City, CA 94404
(415) 358-9600, Extension 345